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Olfactory and Behavioral Mechanisms Underlying Enhanced Mating Competitiveness Following Exposure to Ginger Root Oil and Orange Oil in Males of the Mediterranean Fruit Fly, *Ceratitis capitata* (Diptera: Tephritidae)

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Males of the Mediterranean fruit fly, Ceratitis capitata*, are strongly attracted to various plant odors, and previous work has demonstrated that male exposure to certain odors, including the scent of orange oil (OO) and ginger root oil (GRO), increases their mating success relative to non-exposed males. However, the mechanism(s) underlying this mating increase is not known. Here, we describe several experiments that further investigate the association between GRO- and OO-exposure and male signaling activity, pheromone attractiveness, and mating success in male medflies. Exposure to GRO or OO increased time spent pheromone calling but did not accelerate the rate of male sexual maturation. Using a wind tunnel, we compared female attraction to the pheromone of control, non-exposed males versus males previously exposed to OO or GRO. There was no evidence that GRO exposure enhanced the attractiveness of the male pheromone. The data for OO were inconclusive: females tended to spend more time on spheres emanating pheromone from OO-exposed males than on spheres emanating pheromone from non-exposed males, but the number of female landings did not differ between the*

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two types of pheromone sources. Female choice tests confirmed that GRO- and OO-exposure boost male mating success relative to non-exposed males. Application of GRO directly to the abdomen reduced male mating success, whereas similar application of OO boosted male mating success. The potential role and mode of action of plant chemicals in the mating behavior of male medflies are evaluated in light of these findings.

KEY WORDS: Mediterranean fruit fly; *Ceratitis capitata*; orange oil; ginger root oil; pheromone; mating success.

INTRODUCTION

Among males of some herbivorous insect species, plant-borne chemicals may influence pheromone composition and/or production, and the pheromonal signal may, in turn, influence male mating success (Landolt and Phillips, 1997). Particular plant chemicals ingested by larvae (Lofstedt *et al.*, 1989; Stennett and Etges, 1997) or adults (Pliske, 1975; Krasnoff and Dussourd, 1989) may serve as precursors for pheromone synthesis. Host plant volatiles may also trigger pheromone release in males (Jaffe *et al.*, 1993) or synergistically increase the attractiveness of the male sex pheromone signal to females (Dickens *et al.*, 1990; Landolt *et al.*, 1994).

Plant compounds appear to have a profound influence on the mating behavior of certain tephritid fruit flies, including several species of the genus *Bactrocera* as well as the Mediterranean fruit fly, *Ceratitis capitata* (Metcalf, 1990; Light and Jang, 1996; Landolt and Phillips, 1997). In particular, exposure to certain plant compounds has been shown to increase male mating success in several species. For example, males of the Oriental fruit fly, *B. dorsalis*, are strongly attracted to methyl eugenol, which they ingest and use in synthesizing the sex pheromone (Tan and Nishida, 1996). Males that have consumed methyl eugenol produce a more attractive pheromone than methyl eugenol-deprived males and thereby gain an advantage in mating competition (Shelly and Dewire, 1994). Similarly, increased mating success is evident among *B. dorsalis* males that have fed on flowers of tropical plants containing methyl eugenol (Nishida *et al.*, 1997). In addition, Tan and Nishida (1998) have proposed that methyl eugenol may also confer partial defense against vertebrate predators, such as lizards and birds.

In the Mediterranean fruit fly (or medfly), males are strongly attracted to odors emanating from the peel of orange fruits and the bark of guava trees and readily feed on these substrates (Katsoyannos *et al.*, 1997; Shelly and Villalobos, 2004). Analogous to the situation described above for *B. dorsalis*, males of *C. capitata* exposed to these substrates have increased mating success relative to males deprived exposure (Papadopoulos *et al.*,

2001; Shelly and Villalobos, 2004; Shelly *et al.*, 2004). However, in contrast to *B. dorsalis*, the identity of the compound(s) responsible for this effect and its mode of action are unknown. Data suggest that the hydrocarbon sesquiterpene a-copaene, a powerful attractant to male medflies (Flath *et al.*, 1994a,b) present in both orange and ginger root oil, is important as males exposed to pure a-copaene had a higher mating frequency than non-exposed males (Shelly, 2001). This finding, of course, does not eliminate the possibility that other compounds, either acting individually or collectively, may produce a similar response.

In addition to our lack of knowledge regarding the chemical compounds involved, the olfactory and/or behavioral mechanism(s) responsible for enhanced male mating performance has not been elucidated in the medfly. Male medflies display lek behavior and defend individual leaves as mating territories (Prokopy and Hendrichs, 1979). While perching on the leaf undersurface, males evert their rectal epithelium and emit a sex pheromone attractive to females (Arita and Kaneshiro, 1986). Several lines of evidence suggest that, unlike *B. dorsalis* males, male medflies do not use specific plant chemicals to produce "high quality" pheromone. First, neither a-copaene nor structurally related compounds are present in the pheromone of *C. capitata* males . Second, tests using commercially available oils (and not natural substrates) revealed that exposure to α-copaene-containing ginger root oil or orange oil (hereafter referred to as GRO and OO, respectively) increased mating success in male medflies even when the treated males simply perch close to the oil but are prevented from ingesting it (Papadopoulos *et al.*, 2001). Third, field data (Shelly, 2001) indicate that GRO exposure, while resulting in an increase in the time spent pheromone-calling, does not increase the attractiveness of the pheromone signal itself. Although GRO-exposed males attracted more females than non-exposed males, this difference could be attributed directly to an increase in signaling effort.

While suggestive, these observations do not conclusively eliminate the possibility that certain plant compounds serve as pheromone precursors for male medflies. For example, as noted above, plant-borne compounds other than a-copaene might be important in pheromone synthesis. Moreover, aerial absorption of pheromone precursors has been documented in bark beetles (Hughes, 1974; Byers, 1982), consequently it is possible that males could sequester certain compounds without feeding directly on GRO or OO. Finally, although the field data did not reveal strong female attraction to the pheromone of GRO-exposed males, the trials were not conducted in a controlled environment, and other factors (e.g., wind direction or distribution of food resources) could have confounded the relationship between GRO-exposure and pheromone attractiveness.

Here, we describe a series of laboratory experiments that further explore the association between GRO- and OO-exposure and signaling activity and pheromone attractiveness in male medflies. Our specific aims were to: (i) confirm that oil exposure increased the time spent pheromone-calling, (ii) determine whether oil exposure accelerated male sexual maturation as indicated by a temporal shift (to younger ages) in the expression of pheromone-calling, (iii) compare female attraction to the odor of oil-exposed versus non-exposed males in a wind tunnel, and (iv) determine whether the odor of oil-exposed males increases female mating propensity. This latter experiment was prompted by a study (Mankin *et al.*, 2000) on *Anastrepha suspensa*, showing that females previously exposed to male sex pheromone were more likely to approach the acoustic, calling song of males than females having no prior exposure to male pheromone. In addition, we performed experiments on mating competitiveness that measured (v) the mating success of GRO- or OO-exposed males when competing against non-exposed males or against one another, and (vi) the effect of topical application (as opposed to airborne exposure) of GRO or OO on male mating success.

METHODS

Study Insects and Exposure Protocol

The experiments were conducted during November–December, 2002, in Honolulu and Hilo, Hawaii. Unless otherwise noted, the flies used were collected from field infested Jerusalem cherries (*Solanum capsicum*) and reared for one year in the laboratory (see Shelly, 2001 for details of rearing methods). Flies were sexed within 24 h of emergence. In most experiments, the flies used (both males and females) were sexually mature (8–12 days old). GRO and OO were obtained from Citrus and Allied Essences Ltd. (Lake Success, NY). In most experiments, males were exposed to either GRO or OO for 3 h one day before testing. Twenty-five μl of the appropriate oil were applied with a micropipette to a piece of blotter paper that was placed in a small Petri dish (6 cm diameter). The Petri dish was placed in a nylon-screened cage (30 cm cube), where 50–100 males were held with food (a sugar-yeast hydrolysate mixture in a 3:1 ratio) and water. Although males were free to contact the oil-laden paper, frequent observation revealed that, as noted previously (Shelly, 2001), males did not perch on or touch the paper but rested nearby in a quiescent state. Flies exposed to the different oils were kept in separate rooms to avoid contamination. Non-exposed males were kept in identical conditions in a separate GRO- and OO-free room.

All flies were maintained at 22–25°C and 65–85% RH and received both natural and artificial lighting during a natural 12:12 (L:D) photoperiod.

Effect of Oil Exposure on Male Sexual Signaling and Sexual Maturation

Upon emergence, groups of 10 males were placed in glass cages (30 cm cubes with a cloth sleeve on one side) and exposed to GRO or OO or were deprived of oil exposure. Following the protocol described above, we exposed males to GRO or OO for 2 h (starting at 1500) when 1, 3, 5, and 7 days old. Each day between male ages 2–8 days we recorded the number of males that were pheromone calling per cage at 10 min intervals from 0800–0950 (the period of peak sexual activity). Eight cages were set up for each of the three treatments. Thus, on a given day, the incidence of pheromone calling was recorded over 96 observations for each treatment per male age group (8 cages $\times$ 12 observations per cage). Food and water were supplied ad libitum, and dead males were replaced with similarly treated individuals of the same age.

Wind Tunnel Response of Females to Male Scent

Female response to male scent was measured in a wind tunnel using flies from a mass-reared colony (Maui Med) maintained since 1994 on a wheat-based artificial diet (Tanaka *et al.*, 1969). The use of mass-reared flies was considered justified, because (i) mass-reared males show the same mating enhancement following oil exposure as wild males (Shelly and Mclnnis, 2001) and (ii) wild females show similar attraction to the pheromone of wild versus mass-reared males , suggesting that the pheromone of mass-reared males is functionally equivalent to that of wild males. The sexes were separated as pupae and maintained in groups of 25 individuals in screen-covered cups (volume 850 ml) containing the sugar-protein hydrolysate mixture and water. Males and females were 6–7 days old when tested.

The response of virgin females to the olfactory signals of oil-exposed versus non-exposed males was compared in a two-choice experiment in a laboratory flight tunnel. The apparatus was 261 cm long $\times$ 85.5 cm wide, and 86.5 cm tall and was constructed of tempered glass on all sides (with the floor covered with white paper) with inlet and exit variable-speed box fans attached to galvanized sheet metal enclosures. A laminar airflow system was established using diagonally placed aluminum screen mesh and a honeycomb of horizontally stacked plastic drinking straws placed in front of the fan on the inlet side of the tunnel. A laminar air flow of 20 cm/s was

maintained in the tunnel during the trials, primarily using the inlet fan to "push" air through the tunnel. The tunnel was evenly lit by 40-W fluorescent lights (25–35 foot candles).

Fifty males of a given type were placed in sealed, glass containers (20.5 cm tall, 17.5 cm diameter) outside of the wind tunnel, and male scent was swept from these chambers to the flight tunnel via Teflon tubing using breathing quality, compressed air (see Jang and Light, 1991 for additional details). Males were exposed to GRO or OO for 3 h 1 day before testing following the above protocol. The odors were flushed into two, yellow, hollow polyethylene spheres (7.5 cm diameter). Each sphere was accompanied by three green, plastic leaves that were positioned 2–4 cm above the sphere. One sphere received odor from the oil-exposed males, while the other received odors from the non-exposed males. [While pheromone calling was not monitored, and may, in fact, have been greater for treated males (see below), it is assumed that any differences in signaling activity between treated and control males would have only a minor effect on female response in the wind tunnel given the large volume of pheromone produced collectively by the large (50 individuals) groups established for both male types]. The spheres were suspended about 30 cm apart from the top of the wind tunnel at the upwind end. Both spheres had been perforated approximately 60 times (using a #19 hypodermic syringe), allowing the odors to emanate into the flight tunnel.

In all trials, fifty females were released from the downwind end of the tunnel, where a cup was introduced through a small door and gently opened, allowing the females to exit freely. Female response was compared under two experimental regimes. Females were presented with (1) the odor from OO-exposed males versus the odor from non-exposed males or (2) the odor from GRO-exposed males versus non-exposed males. For each of these comparisons, we performed two sets of trials. In the first, an observer recorded both the total number of female landings and the total arrestment time (over all landings using a stopwatch) on spheres for 20 min (following the procedure of Jang and Light, 1991). These data were collected for 12 replicates involving GRO-exposure and 20 replicates involving OO-exposure. In the second, we monitored only the number of females landing by coating the two spheres with Tangletrap adhesive (The Tanglefoot Co., Grand Rapids, MI; odor emission holes were left uncovered). These data were gathered for 10 replicates of each experiment. All females were removed from the wind tunnel at the end of a replicate. However, the same two groups of oil-exposed and non-exposed males, respectively, were used for all 5–6 replicates conducted on a given day. The wind tunnel was cleaned at the end of each test day with water. Experiments were conducted between 0900–1300 at 24–26°C and RH 55–65%.

Aphrodisiac Effect of Male Scent on Females

Here, we tested whether the odor of oil-exposed males increases the mating propensity of females. In the afternoon before testing, 10 mature (8–10 day old) virgin females were placed in glass cages (30 cm cubes with a cotton sleeve on one side) with the sugar-yeast hydrolysate mixture and water. The next day at 0700 we introduced two plastic cups (13 cm high, 6 cm base diameter, 10 cm top diameter) each containing five males of a particular treatment (GRO-exposed, OO-exposed, or non-exposed) into the cages (exposure to oil was conducted for 3 h on the preceding day using the standard protocol). The two cups introduced into the same cage contained males of the same treatment category, consequently females were exposed to olfactory cues from 10 GRO-exposed, 10 OO-exposed, or 10 non-exposed males. Both the top and bottom of the cups had been removed and covered with nylon screening. Thus, physical contact between the sexes was prevented. In addition, we established cages of females into which no males at all were introduced.

The two "male cups" were removed from the cages at 0900, and 10 non-exposed males were introduced into the cages. The number of mating pairs was recorded every 10 min until 1100. Eight replicates were run for each of the following treatments: (i) females exposed to odor of GRO-exposed males, (ii) females exposed to odor of OO-exposed males, (iii) females (CE, control experienced) exposed to odor of non-exposed males, and (iv) naive females (CN, control naive) not exposed to any male odor. Exposure and mating were run in separate rooms for each treatment to avoid exposing females to 'mixed' or 'contaminated' signals.

Mating Competitiveness Following Oil Exposure

Two series of experiments were run to assess the effect of oil exposure on male mating success. In the first, we compared the mating success of males exposed to the aroma of GRO or OO, respectively, against non-exposed males and against one another. In all tests, we introduced two males (from two different treatments) and a single female into screen-covered, transparent plastic cups (400 ml) at 0800 and recorded matings during the following 2 h. Competition was investigated between the following male treatments: (i) GRO-exposed versus non-exposed, (ii) OO-exposed versus non-exposed, and (iii) GRO-exposed versus OO-exposed.

As described below, in the latter test OO-exposed males displayed a mating advantage over GRO-exposed males. To confirm this result

under more natural conditions, we repeated this test using field-cages (3 m diameter, 2.5 m height, see Shelly, 2001 for field-cage protocol). Fifty GRO-exposed males, 50 OO-exposed males, and 50 females were introduced in the cages at 0800 (all flies used were mature virgins), and mating pairs were collected over the next 3 h. One day before testing, males were chilled and marked with enamel paint on the thorax for identification. Four cages were run on each of 2 days for a total of 8 replicates.

In the second series of experiment, we examined whether topical application of the oils influenced male mating success. Using a micropipette, we applied a small amount (0.065 μl) of GRO or OO to the dorsal part of the abdomen or the tip of one wing (prior to application, males were chilled at 5°C for 15 min). We anticipated that following abdominal application the active ingredient(s) would be absorbed through the cuticle and be transported by haemolymph to the rectal pheromone-producing glands. In contrast, application of oil to the wing tip would aromatize the male but not allow for absorption into the body. Mating trials were conducted 1 day post-treatment using the same procedures described above, except that all trials involved an oil-treated male versus a control male (i.e., no tests were run comparing GRO- versus OO-exposed males).

RESULTS

Male Signaling and Sexual Maturation

Exposure of males to GRO and OO significantly increased signaling activity (Fig. 1). Repeated measures ANOVA (using data from days 3–8) revealed that both day ($F_{5,60} = 60.9$, $P = 0.01$) and exposure ($F_{2,12} = 4.4$, $P = 0.04$) had a significant effect on signaling. The number of males observed signaling (an index of the time spent signaling) was higher in oil-exposed males than non-exposed males every day between days 3–8 (LSD test, $P < 0.05$ in all tests). However, the interaction between treatment and the repeated factor (day) was not significant ($F_{10,60} = 1.2$, $P = 0.27$), indicating that exposure to oils increased the frequency of signaling but not the age of maturation. Overall, there was no significant difference between GRO- and OO-exposed males (LSD test, $P = 0.21$), however, both GRO- and OO-exposed males exhibited significantly higher signaling activity than non-exposed males (LSD test, $P = 0.01$ and $P = 0.001$, respectively).

Response of Females to Male Scent

In the wind tunnel trials using non-sticky spheres, we found no significant difference between spheres emanating odors from GRO-exposed

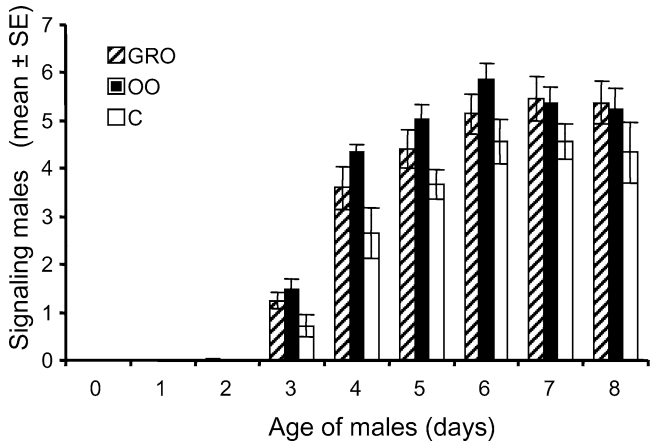

Fig. 1. Effect of exposure to ginger root oil (GRO) or orange oil (OO) on signaling intensity and sexual maturation of *C. capitata* males relative to non-exposed, control (C) males. Bar height represents mean (± 1 SE) number of signaling (pheromone calling) males per observation for a given age. Eight replicates (cages) each involving 10 males were run per treatment, with signaling scored every 10 min over a 2 h period per replicate ($n = 96$ observations per age group).

males versus non-exposed males in either the total number of female landings ($t = 0.4$, $df = 11$, $P = 0.7$) or total female arrestment time ($t = 0.5$, $df = 11$, $P = 0.65$; Fig. 2). Likewise, the total number of female landings did not differ significantly ($t = 0.9$, $df = 19$, $P = 0.4$) between spheres emanating odor from OO-exposed males versus non-exposed males (Fig. 2). However, the total female arrestment time was significantly greater on spheres associated with OO-exposed males than control, non-exposed males ($t = 2.3$, $df = 19$, $P = 0.03$; Fig. 2).

In the wind tunnel trials using sticky spheres, similar numbers of females were captured on spheres emanating pheromone from control males or from males exposed to GRO (means ± 1 SE: 4.05 ± 0.3 versus 4.65 ± 0.43, respectively, $t = 0.4$, $df = 9$, $P = 0.6$) or OO (4.66 ± 0.42 versus 4.02 ± 0.26, respectively, $t = 0.5$, $df = 9$, $P = 0.3$).

Aphrodisiac Effect of Male Scent

Exposure to the odor of GRO-exposed, OO-exposed, or non-exposed males increased female mating propensity slightly, but not significantly, relative to females that had no exposure to any male scent at all ($F_{3,28} = 1.2$,

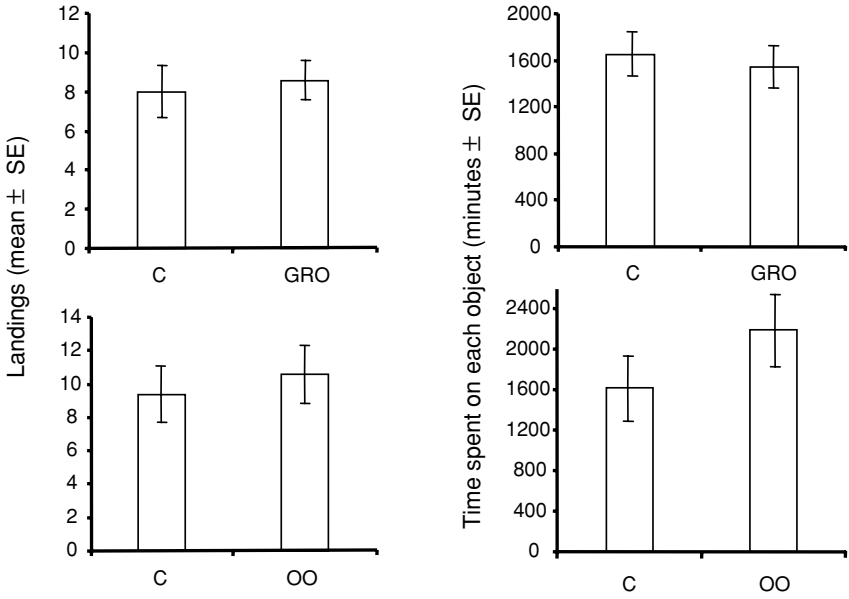

Fig. 2. In trials using non-sticky spheres, wind tunnel response (landings – left column, total cumulative arrestment – right column) of virgin females to pheromone emitted from (top graphs) males exposed to ginger root oil (GRO) or non-exposed males (C) and (bottom graphs) males exposed to orange oil (OO) or non-exposed males (C). Fifty females were released into the wind tunnel for each replicate, and 50 males were used in each treatment. A total of 12 and 20 replicates were conducted for the GRO and OO trials, respectively.

$P = 0.3$). The mean numbers (± 1 SE) of matings observed for females with the different exposure regimes were: GRO – 6.25 ± 0.62, OO – 6.87 ± 0.35, CE – 6.75 ± 0.62, and CN – 5.5 ± 0.66 ($n = 8$ replicates for each treatment with 10 females observed per replicate).

Male Mating Competitiveness

In the first experiment, OO- and GRO-exposed males obtained significantly more matings than non-exposed, control males (Table I), with males in both treatments achieving the same high proportion (59%) of the total matings ($G = 0.02$, $P > 0.05$, G test with Yates correction). In addition, OO-exposed males had a significant mating advantage in direct competition with GRO-exposed males (61% versus 39% of matings, respectively; $Z = 2.2$, $n = 46$, $P < 0.05$). However, when this latter test was repeated in field-cages, we found no significant difference between the number

Table I. Results of Mating Experiments Comparing OO- or GRO-Exposed (Treated) Males and Non-Exposed (Control) Males

	Percentage of matings			
	Treated	Control	*N*	*Z*
Orange oil (OO)				
Aroma	59.0	41.0	100	3.2**
Topical				
Abdomen	61.0	39.0	41	2.0*
Wing	56.4	43.6	39	0.6 NS
Ginger root oil (GRO)				
Aroma	58.9	41.1	112	3.6***
Topical				
Abdomen	40.8	59.2	71	2.4*
Wing	55.0	45.0	40	0.4 NS

Note. Different treatments included exposure to the oil's aroma or topical application of the oil to abdomen or wings. Significance was determined using normal approximation of the binomial distribution (Z test) with the following designations: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, NS: $P > 0.05$.

of matings achieved by GRO- versus OO-exposed males (mean ± 1 SE: 14.7 ± 1.0 versus 14.2 ± 1.4, respectively, $t = 0.2$, $df = 14$, $P > 0.05$).

Application of OO to the abdomen of males significantly increased their mating success, while application of GRO to the abdomen significantly reduced male mating success (Table I). For both oils, application to the wing had no apparent effect on male mating frequency (Table I).

DISCUSSION

The present study confirms previous research showing that exposure to essential oils, specifically OO (Papadopoulos *et al.*, 2001; Shelly *et al.*, 2004) and GRO (Shelly, 2001), increases the mating success of male medflies. These two oils produced a similar increase in male performance: males exposed to OO or GRO obtained the same proportion of total matings (59%) when competing against non-exposed, control males. Despite the similarity in their performance against non-exposed males, OO-males had a significant mating advantage over GRO-exposed males in trials involving direct competition between these treatments. However, OO- and GRO-exposed males had equivalent mating success in trials conducted in field cages.

Regarding the potential behavioral mechanism(s) underlying the oil-mediated boost in mating success, the present study confirmed previous results for GRO (Shelly, 2001) and demonstrated for the first time for OO that exposure to these oils significantly increases the signaling activity

of male medflies. Previous work has documented a direct relationship between signaling activity and mating frequency in male medflies (independent of any chemical exposure), and therefore it is likely that an oil-mediated increase in male signaling contributed to the enhanced male mating success reported in the present study. GRO and OO apparently have a similar effect on signaling behavior as the number of pheromone calling males recorded per observation did not differ significantly between OO- and GRO-exposed males (Fig. 1). Based on data from 6–8 day-old males (all of whom were most likely sexually mature), oil exposure increased male signaling activity by approximately 25% (5.4 versus 4.3 signaling males per observation, on average, for oil-exposed and non-exposed males, respectively). Although the protocols differed, this relative increase is very similar to that noted previously for GRO-exposed males under field conditions (Shelly, 2001). Interestingly, increase of mating success for OO- and GRO-exposed males was approximately 20%.

While both GRO and OO boosted signaling activity, the two oils appeared to have different effects on female attraction to male olfactory cues. The wind tunnel experiments provided no evidence that the scent of GRO-exposed males induced more female landings or greater female arrestment than that of control males. These results imply that the increased mating success of GRO-exposed males did not result from any increase in the attractiveness of olfactory cues deriving from this exposure. Moreover, the absence of an aphrodisiac effect following female exposure to the odor of GRO-exposed males further suggests that the increased mating success of such males did not simply reflect an olfactory-induced increase in female mating propensity.

In contrast, data from the wind tunnel revealed an effect of male exposure to OO on female behavior. Although numbers of female landings did not differ between spheres (sticky or non-sticky) emanating the scent of OO-exposed versus control males, the total arrestment time of females (on non-sticky spheres) was significantly greater for OO-exposed males than control males (Fig. 2). Thus, after arriving at an odor source, females apparently distinguished between the scent of OO-exposed versus control males, showing greater arrestment in response to the scent of OO-exposed males. This behavioral tendency may have contributed to the greater mating success of OO-exposed males, because such males would presumably have extra time to court and mount females. As with GRO, exposure to the odor of OO-treated males did not increase female mating propensity, indicating that, while the scent of OO-exposed males acts as an arrestant, it does not increase the mating readiness of females.

It is important to note that the wind tunnel experiments did not distinguish between female response to the ‘body odor’ of males (i.e., cuticular

scent, possibly altered by oil exposure) and the sex pheromone emitted via the rectal epithelium. Thus, in the case of OO-exposed males, the wind tunnel data can not definitively ascribe increased female arrestment to cuticular odor versus pheromone. However, because topical application of OO to the wings of males had no significant effect on their mating success, it appears unlikely that an OO-mediated change in cuticular scent was responsible for the greater female arrestment or mating success of OO-exposed males. The finding that abdominal application, and presumed subsequent absorption, of OO enhanced male mating success further suggests that specific components of OO are used to synthesize a pheromone more likely to arrest females and to facilitate mating. Like OO, GRO applied to the wing had no effect on male mating performance, but unlike OO, GRO applied to the abdomen actually had a negative effect on male performance. The quantity of oil applied was arbitrary and, while effective for OO, it may have exceeded the optimal level for GRO. Evaporation of the small amount of the oil applied on the wings might affect males in a similar way with the standard oil-exposure, and this may account for the slight (not significant though) increase in mating success of the males that were treated with oil on the wing. It is well documented that females are not attracted to OO (Katsoyannos *et al.*, 1997). Therefore, the possibility that females detect the oil on males should be excluded as a possible mechanism of the increasing mating success of the oil exposed males.

While the present findings suggest a minor role for olfactory cues for GRO-exposed males, evidence from another study suggests that female medflies distinguish between the courtship behavior of GRO-exposed and non-exposed males. Analysis of behavioral elements using videotape revealed that females more readily "accept" or "cooperate" with males exposed to GRO than non-exposed, control males (whether male exposure to OO similarly accelerates female acceptance is unknown). The durations of pre-mounting activities, such as wing vibration and buzzing and head rocking, were significantly lower for GRO-exposed than control males. This finding suggests that GRO-exposed males (i) displayed one or more courtship elements in a distinctive form or rate, (ii) displayed 'standard' courtship but emitted distinctive, close-range olfactory cues, or (iii) both of the above.

Additional experiments are clearly needed to assess the possible influence of oil exposure on close-range, olfactory signals. For example, removal of female antennae might elucidate the relative importance of visual and tactile cues versus olfactory cues in mate choice. If antennae-less females exhibit the same preference for oil-exposed males as normal females (or females having only a single antenna removed), then close-range, olfactory cues would appear to be of minor importance in mate selection.

Conversely, if antennal removal generates random mating, then male scent would indeed appear to be a major influence on female choice. Also, female response to the solvent (hexane or acetone) extract of oil-exposed versus non-exposed males applied to decoys (a technique frequently used to demonstrate male response to female cuticular compounds, e.g., Huyton *et al.*, 1980; Tregenza and Wedell, 1997) or to non-exposed males following close confinement with oil-exposed males (thereby examining potential transfer or 'rub-off of cuticular scents, e.g., Uebel *et al.*, 1975; Marcillac and Ferveur, 2004) may also help assess the importance of cuticular odor in medfly mating.

In conclusion, it should be noted that, as with the proximate mechanisms of mate selection, uncertainty exists regarding the ultimate, or evolutionary, basis of female preference for oil-exposed males. Based on data from GRO-exposed males, there is no evidence that oil-exposed males confer higher direct fitness benefits than non-exposed males. Female fecundity and longevity, egg hatch, and egg-to-pupal development were similar between females mated to GRO-exposed versus non-exposed males (Shelly, 2005; comparable data are not available for OO-exposed males). Alternatively, the scent of oil-exposed males may indicate a superior ability to locate natural sources of a-copaene or other important compounds in the environment. By selecting exposed males, females may increase the chances that their sons will have a high ability to locate sources of a-copaene and hence enjoy high mating success (i.e., this may be a case of runaway selection, Andersson, 1994). Finally, female preference for exposed males could represent a "sensory trap" (West-Eberhard, 1984) or "sensory exploitation" (Ryan, 1990), where the olfactory signal of exposed males exploits a preexisting bias in females that evolved in a different context, such as searching for food or oviposition resources.

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REFERENCES

Andersson, M. (1994). *Sexual Selection*, Princeton University Press, Princeton, New Jersey.

Arita, L. H., and Kaneshiro, K. Y. (1986). Structure and function of the rectal epithelium and anal glands during mating behavior in the Mediterranean fruit fly male. *Proc. Hawaiian Entomol. Soc.* **26:** 27–30.

Briceno, R. D., Eberhard, W. G., and Shelly, T. E. (2005). Male courtship behavior in medflies that have received aromatherapy (Diptera: Tephritidae: *Ceratitis capitata*). Fla. Entomol. in review.

Byers, J. A. (1982). Male-specific conversion of the host plant compound, myrcene, to the pheromone, (+)-ipsdienol, in the bark beetle, *Dendroctonus brevicomis*. *J. Chem Ecol.* **8:** 363–371.

Dickens, J. C., Jang, E. B., Light, D. M., and Alford, A. R. (1990). Enhancement of insect pheromone responses by green leaf volatiles. *Naturwissenschaften* **77:** 29–31.

Flath, R. A., Cunningham, R. T., Mon, T. R., and John, J. O. (1994a). Additional male Mediterranean fruit fly (*Ceratitis capitata* Wied.) attractants from angelica seed oil *(Angelica archangelica* L.). *J. Chem. Ecol.* **20:** 1969–1984.

Flath, R. A., Cunningham, R. T., Mon, T. R., and John, J. O. (1994b). Male lures for Mediterranean fruit fly (*Ceratitis capitata* Wied.): Structural analogues of α-copaene. *J. Chem. Ecol.* **20:** 2595–2609.

Hughes, P. R. (1974). Myrcene: A precursor of pheromones in *Ips* beetles. *J. Insect Physiol.* **20:** 1271–1275.

Huyton, P. M., Langley, P. A., Carlson, D. A., and Coates, T. W. (1980). The role of sex pheromones in inhibition of copulatory behaviour by male tsetse flies, *Glossina morsitans morsitans*. *Physiol. Entomol.* **5:** 243–252.

Jaffe, K., Sanchez, P., Cerda, H., Hernandez, J. V., Jaffe, R., Urdaneta, N., Guerra, G., Martinez, R., and Miras, B. (1993). Chemical ecology of the palm weevil *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae): Attraction to host plants and to a male produced pheromone. *J. Chem. Ecol.* **19:** 1703–1720.

Jang, E. B., and Light, D. M. (1991). Behavioral responses of female oriental fruit flies to the odor of papayas at three ripeness stages in a laboratory flight tunnel (Diptera: Tephritidae). *J. InsectBehav.* **4:** 751–762.

Katsoyannos, B. I., Kouloussis, N. A., and Papadopoulos, N. T. (1997). Response of *Ceratitis capitata* to citrus chemicals under semi-natural conditions. *Entomol. Exp. Appl.* **82:** 181–188.

Krasnoff, S. B., and Dussourd, D. E. (1989). Dihydropyrrolizidine attractants for arctiid moths that visit plants containing pyrrolizidine alkaloids. *J. Chem. Ecol.* **15:** 47–60.

Landolt, P. J., and Phillips, T. W. (1997). Host plant influences on sex pheromone behavior of phytophagous insects. *Annu. Rev. Entomol.* **42:** 371–391.

Landolt, P. J., Heath, R. R., Millar, J. G., Davis-Hernandez, K. M., Dueben, D. B., and Ward, K. E. (1994). Effects of host plant, *Gossypium hirsutum* L., on sexual attraction of cabbage looper moths, *Trichoplusia ni* (Huber) (Lepidoptera: Noctuidae). *J. Chem. Ecol.* **20:** 2959–2974.

Light, D. M., and Jang, E. B. (1996). Plant volatiles evoke and modulate tephritid behavior. In McPheron, B. A., and Steck, G. J. (eds.), *Fruit Fly Pests: A World Assessment of Their Biology and Management*, St. Lucie Press, Delray Beach, FL, pp. 123–133.

Lofstedt, C., Vickers, N. J., Roelofs, W. L., and Baker, T. C. (1989). Diet related courtship success in the Oriental fruit moth, *Grapholita molesta* (Tortricidae). *Oikos*, **55:** 402–408.

Mankin, R. W., Petersson, E., Epsky, N. D., Heath, R. R., and Sivinski, J. (2000). Exposure to male pheromones enhances *Anastrepha suspensa* (Diptera: Tephritidae) female response to male calling song. *Fla. Entomol.* **83:** 411–421.

Marcillac, F., and Ferveur, J. F. (2004). A set of female pheromones affects reproduction before, during and after mating in *Drosophila*. *J. Exp. Biol.* **207:** 3927–3933.

Metcalf, R. L. (1990). Chemical ecology of Dacinae fruit flies (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* **83:** 1017–1030.

Millar, J. G. (1995). An overview of attractants for the Mediterranean fruit fly. In Morse, J. G., Metcalf, R. L., Carey, J. R., and Dowell, R. V. (eds.), *The Mediterranean Fruit Fly in*

California: Defining Critical Research, University of California, Riverside, CA, pp. 123–143.

Nishida, R., Shelly, T. E., and Kaneshiro, K. Y. (1997). Acquisition of female-attracting fragrance by males of the oriental fruit fly from a Hawaiian lei flower, *Fagraea berteriana*. *J. Chem. Ecol.* **23:** 2275–2285.

Papadopoulos, N. T., Katsoyannos, B. L, Kouloussis, N. A., and Hendrichs, J. (2001). Effect of orange peel substances on mating competitiveness of male *Ceratitis capitata*. *Entomol. Exp. Appl.* **99:** 253–261.

Pliske, T. E. (1975). Courtship behavior and use of chemical communication by males of certain species of ithomiine butterflies (Nymphalidae: Lepidoptera). *Ann. Entomol. Soc. Am.* **68:** 935–942.

Prokopy, R. J., and Hendrichs, J. (1979). Mating behavior of *Ceratitis capitata* in field cages on host trees. *Ann. Entomol. Soc. Am.* **72:** 642–648.

Ryan, M. J. (1990). Sexual selection, sensory systems and sensory exploitation. *Oxford Surv. Evol. Biol.* **7:** 157–195.

Shelly, T. E. (2005). Does mating with ginger root oil-exposed males confer fitness benefits to female Mediterranean fruit flies, *Ceratitis capitata* (Diptera: Tephritidae)? *Proc. Hawaiian Entomol. Soc.* in press.

Shelly, T. E. (2001). Exposure to a-copaene and a-copaene-containing oils enhances mating success of male Mediterranean fruit flies. *Ann. Entomol. Soc. Am.* **94:** 497–502.

Shelly, T. E. (1999). Trimedlure and the mating competitiveness of irradiated male Mediterranean fruit flies (Diptera: Tephritidae). *Environ. Entomol.* **28:** 780–786.

Shelly, T. E., and Villalobos, E. M. (2004). Host plant influence on the mating success of male Mediterranean fruit flies: Variable effects within and between individual plants. *Anim. Behav.* **68:** 417–426.

Shelly, T. E., Dang, C., and Kennelly, S. (2004). Exposure to orange (*Citrus sinensis* L.) trees, fruit, and oil enhances mating success of male Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]). *J. Insect Behav.* **17:** 303–315.

Shelly, T. E., and Mclnnis, D. O. (2001). Exposure to ginger root oil enhances mating success of irradiated, mass-reared males of Mediterranean fruit fly (Diptera: Tephritidae). *J. Econ. Entomol.* **94:** 1413–1418.

Shelly, T. E., and Dewire, A. L. (1994). Chemically mediated mating success in male oriental fruit flies (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* **87:** 375–382.

Stennett, M. D., and Etges, W. J. (1997). Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. III. Epicuticular hydrocarbon variation is determined by use of different host plants in *Drosophila mojavensis* and *Drosophila arizonae*. *J. Chem. Ecol.* **23:** 2803–2824.

Tan, K. H., and Nishida, R. (1996). Sex pheromone and mating competition after methyl eugenol consumption in the *Bactrocera dorsalis* complex. In McPheron, B. A., and Steck, G. J. (eds.), *Fruit Fly Pests: A World Assessment of Their Biology and Management*, St. Lucie Press, Delray Beach, FL, pp. 147–153.

Tan, K. H., and Nishida, R. (1998). Ecological significance of male attractant in the defence and mating strategies of the fruit fly, *Bactrocera papayae*. *Entomol. Exp. Appl.* **89:** 155–158.

Tanaka, N. L., Steiner, L. F., Ohinata, K., and Okamoto, R. (1969). Low-cost larval rearing medium for mass production of oriental and Mediterranean fruit flies. *J. Econ. Entomol.* **62:** 967–968.

Tregenza, T., and Wedell, N. (1997). Definitive evidence for cuticular pheromones in a cricket. *Anim. Behav.* **54:** 979–984.

Uebel, E. C., Sonnet, P. E., Miller, R. W., and Beroza, M. (1975). Sex pheromone of the face fly, *Musca autumnalis* De Geer (Diptera: Muscidae). *J. Chem. Ecol.* **1:** 195–202.

West-Eberhard, M. J. (1984). Sexual selection, competitive communication and species-specific signals in insects. In Lewis, T. (ed.), *Insect Communication*, Academic Press, New York, pp. 283–324.